

present in equine blood for enabling diagnosis of equine babesiasis. Thus, the present invention, in the fifth aspect, relates to a method for diagnosing equine babesiasis which comprises specifically detecting anti-*Babesia caballi* antibodies present in equine blood by using said recombinant protein as an antigen.

The present invention, in the sixth aspect, relates to a method for diagnosing equine babesiasis which comprises specifically detecting the presence of *Babesia caballi* merozoite in equine blood by using the antibody according the present invention.

A method for diagnosing equine babesiasis may be performed with ELISA, immunochromatography, agglutination, etc.

Patents, publications and literatures cited therein are all incorporated herein for reference.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a photograph of confocal laser microscopic image showing reactivity of the monoclonal antibody BC11D of the present invention with *Babesia caballi*.

Fig. 2 schematically illustrates construction of pGEX/BC48 wherein cDNA clone BC48 is incorporated that has the nucleotide sequence shown in SEQ ID NO: 1 and encodes a 48kDa protein of rhoptry of BC merozoite.

Fig. 3 is a photograph indicating Western blot analysis that shows reactivity between proteins expressed from lysogenic bacteria of phage clone BC48 and the monoclonal antibody BC11D recognizing the BC merozoite 48kDa protein.

BEST MODE FOR CARRYING OUT THE INVENTION

The gene of the present invention encoding a 48kDa protein of rhoptry of BC merozoite may be obtained, for instance, as described hereinbelow. That is, BC-infected erythrocytes with about 10% of a rate of parasite within erythrocytes are prepared by *in vitro* culture as described by Avarzed et al. [J. Vet. Med. Sci. 59(6), 479-481 (1997)]. Total RNAs are then extracted by guanidinium-phenol-chloroform procedure as described by Chomczynski et al. [Anal. Biochem. 162, 156-159 (1987)]. mRNAs are isolated and purified with oligotex-dT 30 (manufactured by Takara K.K.) and cDNAs are synthesized with the mRNAs using Zap-cDNA synthesizer kit (manufactured by Stratagene Inc.). The obtained cDNAs are inserted into λ Zap II phage vector (manufactured by Stratagene Inc.), packaged with Gigapack III packaging system (manufactured by Stratagene Inc.) to construct a cDNA library. The obtained cDNA library is screened immunologically using monoclonal antibody recognizing the 48kDa protein of BC merozoite to give a desired cDNA clone, which is recovered as pBluescript clone

by *in vivo* excision.

The cDNA insert of the thus obtained clone is determined for its nucleotide sequence by, for instance, the dideoxy method by Sanger et al. [Proc. Natl. Acad. Sci. USA, 74, 5463 (1977)]. The nucleotide sequence of the cDNA is consisted of 1,828 base pairs in full length as shown in SEQ ID NO: 1 and contains a structural gene of 1,374 base pairs in full length corresponding to the amino acid sequence of a 48kDa protein of BC merozoite as shown in SEQ ID NO: 1 or 2. The thus obtained cDNA directly or after being modified at its 5' end is inserted into the known expression vector at downstream of promoter by the conventional procedure. The expression vector with the inserted cDNA is then introduced into known cells such as *E. coli*, yeast, animal cells or insect cells by the conventional procedure.

INDUSTRIAL APPLICABILITY

In accordance with the present invention, it is possible to produce stably a 48kDa protein of rhoptry of *Babesia caballi* merozoite as well as a gene encoding said protein in a large amount by the recombinant DNA technique. A protein obtained from the gene of the present invention or from cells wherein said gene is introduced, or a polypeptide constituting a portion of said protein, may be used as an antigen for detecting anti-merozoite antibodies